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EXHAUST POLLUTANTS EFFECTS ON PHOTOSYNTHESIS OF PONDEROSA PINE SEEDLINGS

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FINAL REPORT

to

ROCKY MOUNTAIN FOREST AND RANGE EXPERIMENT STATION

on

EXHAUST POLLUTANTS EFFECTS ON PHOTOSYNTHESIS OF PONDEROSA PINE SEEDLINGS

By

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ABSTRACT

Ponderosa pine (*Pinus ponderosa* Laws.) seedlings were exposed in an environmental chamber to nitrogen dioxide (NO_2) and ozone (O_3) at concentrations of 0.00, 0.01, 0.10, and 1.0 ppm of each gas in one-hour fumigations. Changes in apparent photosynthetic rates and dark respiration rates caused by the presence of each gas alone and to combinations of both gases were measured. One-hour exposures to O_3 resulted in somewhat depressed photosynthetic rates, while NO_2 stimulated dark respiration rates. Combinations of NO_2 and O_3 often were antagonistic, reducing the effect that O_3 had on apparent photosynthesis. This would suggest that short durations of low concentrations of NO_2 and O_3 would have little affect on productivity of ponderosa pine seedlings.

INTRODUCTION

Increasing urbanization and recreational use of mountain forested areas is resulting in growing exposure of trees to air polluted with automobile exhaust fumes. Past research indicates that some tree species react adversely to levels of air pollution already being reached in some forest areas (Barnes, 1972; Botkin et al., 1972; Miller, 1973). With increased vehicular usage within forests, air pollution levels near roadways can be expected to increase. Common pollutants emitted from operation of internal combustion engines include oxides and hydrocarbons. In the presence of ultraviolet radiation, these pollutants can react to form oxidants such as ozone and peroxyacyl nitrate. Oxidant air pollutants, primarily ozone, have been found to be responsible for the chlorotic decline disease reported in the San Bernardino National Forest (Miller et al., 1963, 1969; Parmeter and Miller, 1968; Richards et al., 1968). Continuous air sampling during the growing season for a five-year period indicated that oxidant concentration often exceeded 0.10 ppm in the stands of declining ponderosa pine (*Pinus ponderosa* Laws.)(Miller, 1973). Smog-injured ponderosa pine trees were more susceptible to beetle attack (Cobb and Stark, 1970) and evidence suggested that increase of susceptibility might involve reduced rates of resin flow and rapid rates of resin crystallization in smog-injured trees (Cobb et al., 1968).

Hill and Bennett (1970) found that nitrogen oxides inhibited apparent photosynthesis of oats (*Avena sativa* L.) and alfalfa (*Medicago sativa* L.) at concentrations below those required to cause visible injury. They found an apparent threshold concentration of about 0.6 ppm for the nitrogen oxide pollutants. An additive effect of depressing apparent photosynthesis occurred when plants were exposed to a mixture

of nitrogen oxide and nitrogen dioxide.

Miller et al. (1969) found three-year-old ponderosa pine seedlings fumigated in controlled environment chambers with ozone at 0.15, 0.30, or 0.45 ppm had apparent photosynthetic rates reduced by 10, 70, and 85 percent, respectively, after 30 days of exposure. White et al. (1974) found reductions in apparent photosynthesis of alfalfa caused by two-hour fumigations with combinations of sulfur dioxide and nitrogen dioxide. The degree of synergism decreased as the concentrations of the two gases were increased.

Tingey et al. (1973) found that plant height, production, and shoot-to-shoot ratios were significantly reduced by 5 ppm ozone plus 5 ppm sulfur dioxide in an experiment with soybeans (*Glycine max* (L.) Merr.). The growth reductions resulting from the ozone and sulfur dioxide mixtures were greater than the additive effects of each concentration of the gases, indicating a synergistic effect.

Tingey et al. (1971) exposed six plant species for four hours to nitrogen dioxide, sulfur dioxide, and a combination of the two gases in greenhouse exposure chambers. Although concentrations of nitrogen dioxide below 2 ppm and concentrations of sulfur dioxide below 0.5 ppm caused no leaf injury, injury did develop when plants were exposed to mixtures of 0.05 to 0.25 ppm of each of the two gases. The concentrations of nitrogen dioxide plus sulfur dioxide which caused plant injury were similar to those found in urban areas and might result in reduced yields for plants growing under field conditions.

Ozone (O_3) and nitrogen dioxide (NO_2) occur together in photochemical air pollution, but little is known about how such a combination might affect plants (Reinert, Heagle and Heck, 1975). Therefore, the

objective of this study was to determine the effects of short durations of four concentrations of these two air pollutants on the carbon dioxide (CO_2) exchange of ponderosa pine seedlings. Although chronic injury of ponderosa pine resulting from intermittent exposures to air pollutants has been reported, little information presently exists to assess the physiological effects caused by low concentrations of air pollutants. Subtle physiological effects caused by air pollutants, such as increased respiration rates or decreased photosynthetic rates, may be important in reducing forest productivity.

METHODS AND MATERIALS

Three-year-old ponderosa pine seedlings used in this study were obtained from the nursery of the Colorado State Forest Service in a dormant state and planted in #10 potting cans (volume, 3.28 liters). The potting medium was composed of equal parts (volume/volume) of typical ponderosa pine forest duff and alluvium soil from the same area. The potting medium was not sterilized prior to transplanting the seedlings. Tap water and Hoagland's nutrient solution (Appendix A) were supplied at regular intervals during primary and secondary needle growth. The seedlings were grown in a greenhouse environment at approximately 20°C. Supplemental incadescent lights were used during the dark period. Soil water stress was reduced by regular watering. A pre-conditioned period of 48 hours in a growth chamber (temperature 28°C; photoperiod 24 hours; soil water 0 to 1 bar) was used just prior to measurements of carbon dioxide exchange rates. Seedlings had been growing 35 to 45 weeks in the greenhouse prior to experimentation.

A closed system of gas exchange designed by Ronco (1969) was used in the study. The environment of this closed chamber system was held

constant (temperature 28°C; irradiance 0.00 or > 1.01 ly · min^{-1}; soil water 0 to 1 bar) during photosynthetic and dark respiration determinations. Carbon dioxide (CO_2) exchange was monitored using an infrared gas analyzer (IRGA). Known concentrations of CO_2 were used to calibrate the IRGA. Data on CO_2 concentrations within the system were recorded on a strip chart recorder. Carbon dioxide exchange rates for each determination were determined between 288 and 337 ppm. The seedlings were sealed at the soil surface with a mixture of 1:1 and 1:3 parafin and mineral oil in two thin layers to eliminate soil respiration effects. The three drain holes in each can were sealed with plastic tape.

Photosynthetic and dark respiration rates were determined for each seedling prior to fumigation. Then, fumigation of each seedling was accomplished by removing the seedling from the CO_2 exchange system and placing it into an open system plexiglass assimilation chamber with environmental conditions somewhat similar to those within Ronco's (1969) system. Each seedling was subjected to one pollutant concentration of 0.00, 0.01, 0.10, or 1.00 ppm of either NO_2 or O_3 for a period of one hour. In addition, each possible combination of the above concentrations of the two gases was used to determine if interactions of the two pollutants affected CO_2 exchange rates. A complete factorial arrangement of pollutant concentrations was, therefore, used for the determination of interaction effects. Three replications (seedlings) were used for each treatment.

A Mast ozone meter was used to monitor ozone concentration in the effluent air stream from the fumigation chamber. Ozone was generated by passing air through an ultra-violet light source. Nitrogen dioxide was obtained from a gas cylinder of a predetermined concentration and

monitored in the effluent air stream from the fumigation chamber using a modified Saltzman-Greiss technique as described by Nash (1970).

After treatment the seedlings were returned to the closed chamber system for post-photosynthetic and dark respiration rate determinations. A total of 54 seedlings were tested; nine of these seedlings were fumigated with charcoal-filtered air concentrations of 0.00 ppm of test pollutants.

The pre-treatment and post-treatment photosynthetic and dark respiration rates for each seedling were determined by:

$$\text{mg } CO_2\text{/g ODW/hr} = \frac{44{,}010\ (\Delta \text{ ppm } CO_2\text{/hr})10^{-6}(273)635(33.1917)}{\dfrac{22.414\ (760)(TMP)}{\text{g ODW}}} \qquad (1)$$

where: 44,010 = mg/mole of CO_2

10^{-6} = conversion of ppm from grams

635 = atmospheric pressure (mmHg)

33.1917 = volume of system (ℓ)

22.414 = liters/mole of gas

760 = standard pressure (mmHg)

TMP = temperature of the system (273 + 28°C = 301°K)

g ODW = oven dry weight of seedling needles (g).

All data were analyzed using standard statistical procedures (Steel and Torrie, 1960) with the aid of a CDC-6400 computer using programs from the statistical library (Appendix B).

RESULTS AND DISCUSSION

Apparent photosynthetic rates of ponderosa pine were not affected ($p > .10$) by one-hour fumigations with nitrogen dioxide. However, fumigations with ozone alone did appear to cause a slight depression in apparent photosynthetic rates (Table 1). There was some indication

that NO_2 in combinations with O_3 reduced the effect that O_3 had in depressing photosynthesis. Nitrogen dioxide in combination with O_3 decreased ($p < .05$) dark respiration rates, whereas ozone had little affect on dark respiration rates (Table 2). Interactions among various concentrations of NO_2 and O_3 affected ($p < .01$) both photosynthetic and dark respiration rates when CO_2 exchange data were converted to percentages of controls.

The data suggested that a one-hour exposure time was too short to correlate specific pollutant concentrations to photosynthetic depression or dark respiration stimulation. Previous experiments have used either a continuous fumigation period until depression of photosynthetic rates were noted (Hill and Littlefield, 1969; Thompson, et al., 1970), or fumigation periods were longer than one hour (Bennett and Hill, 1973). This suggests that photosynthetic depression and respiration stimulation threshold levels may be greater than our experimental sensitivity. Several other factors may have also contributed to large variability in the data: variation in the greenhouse air temperature influencing potting soil temperature; needle age differences as suggested by Berry (1974); a great deal of inadvertant handling of the seedlings prior to and during experimentation; and an indeterminable plant resistance to the two pollutants.

Large variability was apparent in the data, with standard deviations consistantly being larger than the means. In addition, a strong correlation existed between the means and the standard deviations. For these reasons, a log transformation was applied to the data. These CO_2 exchange data as a percent of control indicated highly significant ($p < .01$) two-way interactions among the various concentrations of NO_2 and O_3.

While cumulative effects of the two pollutants were noted, individual concentration influences were indeterminable. Combinations of various concentrations of NO_2 with O_3 often were antagonistic, reducing the effect that O_3 had on apparent photosynthesis of ponderosa pine seedlings. These data would suggest that short durations of low concentrations of nitrogen dioxide with ozone would have little affect on productivity of ponderosa pine seedlings.

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LITERATURE CITED

1. Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:133-138.
2. Bennett, J. H. and A. C. Hill. 1973. Inhibition of apparent photosynthesis by air pollutants. J. Environ. Quality. 2:526-530.
3. Berry, C. R. 1974. Age of pine seedlings with primary needles affects sensitivity of ozone and sulfur dioxide. Phytopathology 64:207-209.
4. Botkin, D. B., W. H. Smith, R. W. Carlson and T. L. Smith. 1972. Effects of ozone on white pine saplings; Variation in inhibition and recovery of net photosynthesis. Environ. Pollut. 3:273-289.
5. Cobb, F. W. Jr., D. L. Wood, R. W. Stark and P. R. Miller. 1968. Photochemical oxidant injury and bark beetle infestation of ponderosa pine. Hilgardia 39:127-134.
6. Cobb, F. W. Jr. and R. W. Stark. 1970. Decline and mortality of smog-injured ponderosa pine. J. For. 3:147-149.
7. Hill, A. C. and J. H. Bennett. 1970. Inhibition of apparent photosynthesis by nitrogen oxides. Atmos. Environ. 4:341-348.
8. Hill, A. C. and N. Littlefield. 1969. Ozone, effect on apparent photosynthesis rate of transpiration and stomatal closure in plants. Environ. Sci. and Tech. 3:52-56.
9. Miller, P. R. 1973. Oxidant-induced community change in a mixed conifer forest. Adv. Chem. Series 122:101-117.

10. Miller, P. R., J. R. Parameter, Jr., B. H. Flick and C. W. Martinez. 1969. Ozone dosage response of ponderosa pine seedlings. J. Air Pollut. Control Assn. 19:435-438.

11. Miller, P. R., J. R.Parameter, Jr., O. C. Taylor and E. A. Cardiff. 1963. Ozone injury to the foliage of Pinus ponderosa. Phyto-pathology 53:1072-1076.

12. Nash, T. 1970. An efficient absorbing reagent for nitrogen dioxide. Atmos. Environ. 4:661-665.

13. Parameter, J. R., Jr. and P. R. Miller. 1968. Studies relating to the cause of decline and death of ponderosa pine in southern California. Plant Dis. Rep. 52:707-711.

14. Reinert, R. A., A. S. Heagle and W. W. Heck. 1975. Plant responses to pollutant combinations. p. 159-177. In: J. B. Mudd and T. T. Kozlowski [eds.]. Responses of Plants to Air Pollution. Academic Press, New York. 383 p.

15. Richards, B. L., O. C. Taylor and G. F. Edmunds, Jr. 1968. Ozone needle mottle of pine in southern California. J. Air Pollut. Cont. Assoc. 18:73-77.

16. Ronco, F. 1969. Assimilation chamber for measuring carbon dioxide exchange of tree seedlings in the laboratory. USDA Forest Service Res. Note RM-143. 7 p.

17. Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York. 481 p.

18. Thompson, C. R., E. G. Hensel, G. Kats and O. C. Taylor. 1970. Effects of continuous exposure of navel oranges to nitrogen dioxide. Atmos. Environ. 4:349-355.

19. Tingey, D. T., R. A. Reinert, J. A. Dunning and W. W. Heck. 1971. Vegetation injury from the interaction of nitrogen dioxide and sulfur dioxide. Phytopathology 61:1050-1511.

20. Tingey, D. T., R. A. Reinert, C. Wickliff and W. W. Heck. 1973. Chronic ozone or sulfur dioxide exposures or both affect the early vegetative growth of soybean. Can. J. Plant Sci. 53: 875-879.

21. White, K. L., A. C. Hill and J. H. Bennett. 1974. Synergistic inhibition of apparent photosynthesis rate of alfalfa by combinations of sulfur dioxide and nitrogen dioxide. Environ. Sci. and Tech. 8:574-576.

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Table 2. Average changes (post-treatment minus pre-treatment rates) in dark respiration rates (mg CO_2/g/hr) of ponderosa pine seedlings following a one-hour fumigation with NO_2 and O_3.

Table 1. Average changes (pre-treatment minus post-treatment rates) in apparent photosynthetic rates (mg CO_2/g/hr) of ponderosa pine seedlings following a one-hour fumigation with NO_2 and O_3.

		NO_2(ppm)				
		0.00	0.01	0.10	1.00	Avg O_3
O_3(ppm)	0.00	0.169	0.558	0.606	+0.080[1]	0.313a[2]
	0.01	0.324	0.334	+0.040	0.234	0.213a
	0.10	0.396	+0.040	+0.024	0.266	0.150a
	1.00	0.744	0.276	0.092	+0.038	0.268a
	Avg NO_2	0.408a	0.282a	0.159a	0.096a	

[1]A plus sign indicates increased photosynthetic rate following fumigation.

[2]Means in the same row or column followed by a similar letter are not significantly different at p = 0.05.

Table 2. Average changes (post-treatment minus pre-treatment rates) in dark respiration rates (mg CO_2/g/hr) of ponderosa pine seedlings following a one-hour fumigation with NO_2 and O_3.

		NO_2(ppm)				
		0.00	0.01	0.10	1.00	Avg O_3
O_3(ppm)	0.00	0.258	-0.108[1]	0.768	0.824	0.433a[2]
	0.01	1.216	0.145	0.375	0.316	0.503a
	0.10	0.901	0.207	0.508	-0.099	0.378a
	1.00	1.719	0.679	0.443	-0.309	0.633a
	Avg NO_2	1.023a	0.230b	0.523ab	0.183b	

[1]A minus sign indicates decreased respiration rates following fumigation.

[2]Means in the same row or column followed by a similar letter are not significantly different at p = 0.05.

APPENDIX A

Hoagland's Nutrient Solution

Amount (g/ℓ)	Chemical
236.16	$Ca(NO_3)_2$
101.11	KNO_3
246.48	$MgSO_4$
136.09	KH_2PO_4
38.46	Fe EDTA
2.86	H_3BO_3
1.81	$MnCl_2 \cdot 4H_2O$
0.11	$ZnCl_2$
0.05	$CuCl_2 \cdot 2H_2O$
0.025	$Na_2MoO_4 \cdot 2H_2O$

Appendix B. Raw Data - CO_2 Exchange of Ponderosa Pine

ID	ΔPn	ΔR1	ΔR2	Pn (Resp w/o stems)	R2 (% of control1)	(% of control1)
11003	0.3000	** -0.3300	-0.3949	294.12	50.99	
21001	1.4280	0.8820	1.2307	915.38	412.02	
31002	* -0.0540	-0.9060	-1.1608	34.62	97.73	
12001	0.2580	0.8400	1.0445	165.38	349.68	
22003	1.1820	0.6840	0.8556	1158.82	110.47	
32002	0.3780	0.3120	0.4041	242.31	34.02	
13001	-0.0840	0.1320	0.3888	53.85	138.48	
23002	0.1200	0.3600	0.4309	76.92	36.28	
33003	-0.2760	1.1460	1.6520	270.59	213.30	
10106	-0.4740	0.9180	1.1887	123.44	728.37	
20105	1.0560	1.0980	1.4463	409.30	1323.24	
30104	0.3900	0.7500	1.0138	44.52	369.46	
10204	0.9000	1.3020	1.6558	189.87	603.43	
20205	0.0000	0.0000	0.0000	0.00	0.00	
30206	0.2880	0.8280	1.0473	75.00	641.73	
10306	2.6460	1.0260	1.3758	689.06	843.01	
20304	-1.0920	1.3560	1.6558	124.66	625.26	
30305	0.6780	1.4280	2.1261	262.79	1945.20	
10011	0.1560	0.2400	0.2987	0.00	0.00	
20012	-0.1560	0.9300	1.1878	0.00	0.00	
30013	0.1020	0.5640	0.7745	0.00	0.00	
10024	0.8760	-0.2040	-0.2744	0.00	0.00	
20025	-0.2580	-0.0840	-0.1093	0.00	0.00	
30026	0.3840	-0.1200	-0.1632	0.00	0.00	
10037	0.0000	0.1200	0.1690	0.00	0.00	
20038	0.5760	0.3480	0.4433	0.00	0.00	
30039	-0.1560	0.0000	0.0000	0.00	0.00	
11107	0.3000	0.2340	0.2923	0.00	172.96	
21109	0.5280	-0.1800	-0.2305	338.46	0.00	
31108	0.1740	0.2820	0.3741	30.21	84.39	
11207	0.4200	0.5040	0.7041	0.00	416.63	
21208	0.1140	0.0960	0.1345	19.79	30.34	
31209	-0.6540	-0.1680	-0.2174	419.23	0.00	
11309	0.2100	0.8340	1.2910	134.62	0.00	
21307	0.4440	0.2880	0.3721	0.00	220.18	
31308	0.1740	0.2820	0.3741	30.21	84.39	
12108	-0.2760	0.5760	0.8342	47.92	188.18	
22109	0.0000	0.0000	0.0000	0.00	0.00	
32107	0.1560	0.2220	0.2908	0.00	172.07	
12209	-0.3180	0.2580	0.3443	203.85	0.00	
22208	-0.5100	-0.9420	-1.3997	88.54	315.75	
32207	0.7560	1.6200	2.5782	0.00	1525.56	
12309	0.3900	0.4920	0.6599	250.00	0.00	
22307	0.1140	0.7140	0.9963	0.00	589.63	
32308	-0.2280	-0.2460	-0.3281	39.58	74.01	
13109	-0.3780	0.4260	0.5504	242.31	0.00	
23108	0.0360	0.0600	0.0798	6.25	18.00	
33107	-0.3600	0.2340	0.3179	0.00	188.11	
13207	0.2220	-0.4680	-0.6217	0.00	367.87	
23209	0.6900	0.0480	0.0630	442.31	0.00	
33208	-0.1140	0.1920	0.2627	19.79	59.26	
13307	0.0000	-0.0240	-0.0327	0.00	19.35	
23308	-0.0840	0.2220	0.3371	14.58	76.04	
33309	-0.0300	-0.9660	-1.2322	19.23	0.00	

Control Data

ID = columns 1-5

Col. 1 = Rep. No.

" 2 = $[NO_2]$

" 3 = $[O_3]$

" 4 = Control No.

" 5 = Control used for % of control data

$[NO_2$ or $O_3]$

0 = 0.00 ppm

1 = 0.01 ppm

2 = 0.10 ppm

3 = 1.00 ppm

* Neg. indicates more Pn following treatment

** Neg. indicates more resp. before treatment w/stem

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S P S S - - STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

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RUN NAME         AOV FOR NITTPERSON
VARIABLE LIST    REP,NOL,OL,CO,DPN,DR1,DR2,PCPN,PCR2
INPUT MEDUIM     CARD
N OF CASES       54
INPUT FORMAT     (4F1.0,3X,3(1X,F7.4),2(1X,F7.2))
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ACCORDING TO YOUR INPUT FORMAT, VARIABLES ARE TO BE READ AS FOLLOWS

VARIABLE	FORMAT	RECORD	COLUMNS
REP	F 1. 0	1	1- 1
NOL	F 1. 0	1	2- 2
OL	F 1. 0	1	3- 3
CO	F 1. 0	1	4- 4
DPN	F 7. 4	1	9- 15
DR1	F 7. 4	1	17- 23
DR2	F 7. 4	1	25- 31
PCPN	F 7. 2	1	33- 39
PCR2	F 7. 2	1	41- 47

THE INPUT FORMAT PROVIDES FOR 9 VARIABLES. 9 WILL BE READ
IT PROVIDES FOR 1 RECORDS (*CARDS*) PER CASE. A MAXIMUM OF 47 *COLUMNS* ARE USED ON A RECORD.

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COMPUTE          NANDO = 10 * NOL + OL
VAR LABELS       NOL,NO2 LEVEL/OL,O3 LEVEL/CO,CONTROL NO./DPN,DELTA PN/DR1  ,
                 DELTA R1/DR2,DELTA R2/PCPN,PERCENT CONTROL PN/PCR2,PERCENT
                 CONTROL R2/NANDO,NO2 AND O3 LEVEL/
TASK NAME        MEANS FOR DELTA PN, DELTA R1, DELTA  R2 ,PCT PN , PCT.R2
BREAKDOWN        TABLES = DPN TO PCR2 BY NOL,OL,NANDO
READ INPUT DATA
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GIVEN 1 DIMENSIONS, INITIAL CM ALLOWS FOR 2055 CELLS, MAXIMUM CM ALLOWS FOR 5469 CELLS.